Genetics of ANCA-associated vasculitides

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Within the spectrum of vasculitides, antineutrophil cytoplasmic autoantibodies (ANCA), particularly those directed to proteinase 3 (PR3) and myeloperoxidase (MPO), are strongly associated with the idiopathic pauci-immune small-vessel vasculitides.[1] Data from in vitro and in vivo experimental studies suggest that ANCA are involved in the pathophysiology of those disorders.[2] Although the etiopathogenesis of the ANCA-associated vasculitides has not yet been elucidated, current knowledge strongly points to an autoimmune background of the diseases. It is becoming increasingly clear that genetic factors are involved in the expression of autoimmune diseases. In systemic lupus erythematosus (SLE), multiple genes seem instrumental in disease induction and expression.[3] Also in the ANCA-associated vasculitides, genetic factors may be operative. A number of familial cases have been described,[4-9] but no systematic approach has been undertaken to document increased familial segregation of this group of diseases. The genes supposedly involved in disease induction and expression have not yet been identified for the ANCA-associated vasculitides, but skewing in polymorphisms of both immune response genes and genes encoding for PR3 and its inhibitor alpha-1-antitrypsin (α_1-AT) have been documented. This short report discusses data that point to a role of those particular genes in the ANCA-associated vasculitides.

HLA-GENES

Products of the very polymorphic HLA-genes are involved in immune recognition by T-cells. Class I gene products present peptides to CD8-positive cytotoxic T-cells, and class II gene products are responsible for presentation of antigenic peptides to CD4-positive T-cells. In particular, class II genes are, by their extreme polymorphism, supposed to influence immune responses to specific antigens, both qualitatively and quantitatively. Therefore, associations have been sought between HLA-gene polymorphisms and diseases that are thought to be based on specific autoimmune responses. Also for the ANCA-associated vasculitides many groups have studied possible associations with HLA-genes. Although some negative and positive associations have been found, no consistent and significant associations came out of these studies. In a study on 59 patients, 34 with Wegener's granulomatosis (WG) and 25 with microscopic polyangiitis (MPA), Spencer et al found an increased frequency of HLA-DQ7 and a decreased frequency of HLA-DR3.[10] These findings could not be confirmed by Hagen et al[11] studying 224 patients with ANCA-associated vasculitis. They found a decreased frequency of HLA-DR13DR6, but did not observe differences in the distribution of HLA-antigens between patients with WG and MPA, between anti-PR3- and anti-MPO–positive patients, and between patients with and without relapsing disease. Another study on 37 Greek patients with WG showed a weak association with DR1.[12] Taken together, the data presently available do not point to a specific MHC profile in ANCA-associated vasculitides and, so, not to a particular (exogenous?) antigen involved in disease induction.

OTHER IMMUNE RESPONSE GENES

It has become increasingly clear that not only HLA-genes but almost every gene in an outbred human population shows polymorphism resulting, in many cases, in functionally (slightly) different products. As for genes involved in immuneresponsiveness polymorphisms in genes encoding for Fcγ-receptors (Fcγ-Rs), complement factors C3 and C4, integrins such as CD18, and TNFα have all been studied in the ANCA-associated vasculitides.

As mentioned, ANCA are thought to be involved in the pathogenesis of ANCA-associated vasculitides, mostly by their potential to activate primed neutrophils.[2] This process is clearly dependent on interaction with Fcγ-Rs on neutrophils, although $F(ab')_2$-fragments of ANCA can induce some activation as well. As Fcγ-Rs show functional polymorphism, it is understandable that Fcγ-R polymorphism has been studied in the aforementioned diseases in relation to disease induction and disease expression. As for the individual phenotypes of FcγRIIa, FcγRIIIa, and FcγRIIIb, no significant skewing was observed in patients with WG[13] or ANCA-positive systemic vasculitis,[14-16] although the NA_1 allele of the FcγRIIIb seemed overrepresented in patients with MPO-ANCA–positive systemic vasculitis.[14] Combining haplotype frequencies, it proved that the presence of the homozygous FcγRIIa-H/H131 in combination with the homozygous FcγRIIIa-V/V158 polymorphism constituted a significant risk factor for WG (RR 4.6, CI 1.4-15.2).[13] Individuals with FcγRIIa-H/H131

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bind more IgG3 and are capable of binding IgG2 compared to individuals homozygous for the R/R131 form of FcγRIIa, and individuals with the V/V158 form of FcγRIIIa bind more IgG1 and IgG3 than individuals with the F/F158 form of FcγRIIIa. So, the observed skewing is consistent with a stronger interaction of ANCA with neutrophils. In addition, Dijstelbloem et al[13] showed that WG patients homozygous for both the R131 form of FcγRIIa and the F158 form of FcγRIIIa had an increased risk for relapse of WG (RR 3.3, CI 1.6-6.8). They hypothesized that the presence of these phenotypes with low binding capacity for IgG-subclasses increased the risk for infection or colonization with *Staphylococcus aureus*, a well-known risk factor for relapse.

Persson et al[17] studied complement C3 and C4 allotypes in patients with ANCA-positive vasculitis. Although the role of the complement system in ANCA-positive vasculitis is not as clear as in SLE, immune complexes and complement might be involved in the initial stages of the former diseases.[18] Persson et al found the C3F allele increased in PR3-ANCA–positive patients, but the clinical significance is not clear. They also observed an increased frequency of the C4A3 allele in patients with ANCA-positive vasculitis but, again, the functional significance of this finding is not directly apparent.[17]

Gencik et al[15,19,20] studied a number of other polymorphisms in relationship to immune responsiveness. They detected 10 single nucleotide polymorphisms in the integrin molecule CD18, and 4 of these were associated with MPO-ANCA–positive vasculitis. One of these four was localized in an alternate transcription initiation site and may potentially influence CD18 gene expression and, so, the adhesion cascades of leukocytes. No differences in the distribution of polymorphic phenotypes were detected for a number of other immunoregulatory proteins such as TNFα, IL-2, and IL-5 receptorα.

Also, Huang et al did not find an association of particular polymorphisms of TNFα or IL-1β with WG.[21] They did find an association of a microsatellite of CTLA-4 with WG.[21] As CTLA-4 is involved in T-cell activation, this association is supportive of the role of T-cells in the pathogenesis of WG.

■ POLYMORPHISM IN ALPHA-1-ANTITRYPSIN AND ANCA-ASSOCIATED VASCULITIS

The target antigen of ANCA in WG is PR3 in most of the cases. PR3 is a serine protease from the azurophilic granules of neutrophils released upon degranulation. PR3 is a lytic enzyme and the body is protected against its destructive action by anti-proteases. Amongst these, α_1-AT is of major importance. α_1-AT is encoded by a highly polymorphic gene at the protease inhibitor (Pi) locus. The products are functionally classified as normal (M) or deficient (Z) resulting in, based on its codominant expression, homozygous and heterozygous phenotypes. The Pi MM-variant functions normally, the Pi MZ-phenotype is slightly deficient, and the Pi ZZ-phenotype is severely deficient. One might hypothesize that functional deficiency of α_1-AT results in more severe disease expression or even in induction of anti-PR3 once active PR3 persists in an inflammatory environment. Indeed, Esnault et al[22] observed an increased prevalence of the Pi ZZ and Pi MZ phenotypes in anti-PR3–positive patients. An increased frequency of the Pi Z allele in patients with anti-PR3 has been confirmed by many other groups (reviewed in 23). Reversely, the presence of the Pi Z allele in a random population was not associated with an increased incidence of ANCA-associated vasculitis,[24,25] as far as could be studied in a relatively small population of 47 and 191 individuals being homozygous for Pi ZZ, respectively. Nevertheless, these data show that the Pi ZZ phenotype of α_1-AT is not a major risk factor for ANCA-associated vasculitis but may contribute to disease induction.

■ GENETIC FACTORS RELATING TO PROTEINASE 3 EXPRESSION

PR3 itself, the major target of ANCA in WG, has been shown to be expressed in polymorphic forms. Gencik et al[26] screened the entire coding and promoter sequences of the PR3 gene for polymorphisms. Besides one amino acid change at position 119, they found 7 single-nucleotide polymorphisms, one 84 bp insertion/deletion, and a microsatellite. Comparing PR3 from patients with WG with that of healthy controls they observed the A-564 G polymorphism, localized in the promoter region of PR3, to be overrepresented in WG. The functional significance of this polymorphism is, however, not clear.

Possibly more interestingly, Witko-Sarsat et al demonstrated that PR3, which is mainly localized in the azurophilic granules of PMNs, is present, at least in a subset of PMNs, in the secretory vesicles as well, and can be easily mobilized to the cell membrane.[27,28] The same authors showed that the percentage of PMNs with membrane expression of PR3 on resting cells is constitutively determined for each individual and appears to have a genetic background.[29] Based on family studies they suggest membrane expression of PR3 as a new polymorphism with three different phenotypes: low expression (on <20% of PMNs), intermediate expression (on ±50% of PMNs), and high expression (on ±75% of PMNs).[29]

As stated, membrane expression of PR3 was found present on resting neutrophils. Previous studies had already shown that priming of PMN as well as PMN apoptosis result in surface expression of PR3,[2] a prerequisite for PMN activation by ANCA. However, both "low" and "high PR3 expressing" PMNs can be activated by stimulants such as fmlp, although a comparison has not been made with respect to their, possibly differential, degree of activation by ANCA.

The frequency of the "high expression" phenotype of resting PMNs was increased in patients with systemic vasculitis as well as in patients with rheumatoid arthritis, but not in patients with cystic fibrosis and diabetes, compared to healthy controls.[29] The "high expression" phenotype was not restricted to patients with PR3-ANCA–associated vasculitis but was seen also in MPO-ANCA–positive and ANCA-negative vasculitis patients.[29] The functional significance of this supposed polymorphism is not clear. We recently could confirm increased membrane expression of PR3 on resting PMNs in patients with WG com-

pared to healthy controls, although the difference was of minor significance.[30] When surface expression of PR3 on resting PMN was categorized as low, bi-modal, and high in patients with WG, it proved that bi-modal and high expression was associated with a significantly increased risk for relapse. So, increased membrane expression of PR3 on resting PMN, assumed to be genetically determined, may have functional significance with respect to (ANCA-induced?) neutrophil activation.

CONCLUSION

ANCA-associated systemic vasculitis may develop by the interplay of exogenous and endogenous factors. Within the latter category, genetic factors play a primary role. Genetic factors related to immune response genes seem to be involved as in other autoimmune diseases. In PR3-ANCA–associated vasculitis, genetic polymorphism in the PR3-molecule, as well as in its expression in and on the leukocyte, may influence induction and clinical course of WG, a disease strongly associated with PR3-ANCA. In the same line, functional differences in antiproteases, such as deficiency in α_1-AT, may determine the clinical expression of PR3-ANCA–associated disease. The pathways from genetic risk factors to overt disease still should be elucidated.

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